CCII. THE OXIDATION OF THE FATTY ACIDS *IN VITRO*, WITH ESPECIAL REFERENCE TO THE OXIDATION OF β-HYDROXYBUTYRIC AND ACETOACETIC ACIDS.

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We are still profoundly ignorant of the methods by which the fatty acids are oxidised in the body and of the nature of the intermediate stages of their oxidation: even in the laboratory, the effect of different conditions of oxidation has been little studied. The classic experiments of Dakin [1908] showed that when fatty acids containing six or more carbon atoms in the molecule were oxidised by heating the solutions of their ammonium salts with two molecular proportions of hydrogen dioxide for at least 2 hours on a boiling water-bath, about 10% of the acid underwent β-keto-oxidation, one molecule of CO_2 being split off and the corresponding methylketone formed. The lower acids were rather more completely oxidised, between 40 and 50% of a molecular proportion of CO_2 being split off from formic and propionic and about half that amount from acetic and butyric acids. The identification of propionic and later of as much as 50% of succinic acid [Cahen and Hurtley, 1917] among the oxidation products of butyric acid showed that α-, β- and γ-oxidation of butyric acid had occurred, a similar finding being later established for palmitic acid by the work of Clutterbuck and Raper [1925].

This oxidation of the fatty acids was greatly increased by adding a few drops of a cupric solution to the dioxide reaction mixture [Smedley-MacLean and Battie, 1929]; in the case of the higher fatty acids, evidence was produced that in the presence of the catalyst hydroxylation took place simultaneously at several points in the chain of carbon atoms, unsaturated fatty acids being formed and a considerable proportion of the original acid broken down to acids containing 4 or fewer carbon atoms [Smedley-MacLean and Pearce, 1934].

A quantitative study has now been made of the oxidation of the lower fatty acids by means of hydrogen dioxide and of the effect produced by the addition of the copper catalyst. The earlier experiments were carried out under similar conditions to those already recorded [Smedley-MacLean and Battie, 1929], but this method had the disadvantage that the reaction took place in a medium in which the degree of acidity constantly increased as acid products were formed: further, the concentration of hydrogen dioxide was constantly decreasing since the total amount of the dioxide was added at the beginning of the experiment.

Preliminary experiments.

Preliminary experiments established that when a solution of the sodium salt of a lower fatty acid was treated with a large excess of hydrogen dioxide at 60° in the presence of a cupric salt, the acids were oxidised to CO_2 in the following proportions: formic, 90%; acetic, 30%; propionic, 40%; butyric, 10%.

At 90°, from 20 to 25 % of the total carbon of butyric acid appeared as CO_2, 9–14 % as succinic acid, 4–5 % as acetone, 3 % as formic acid and 3 % as aldehyde. Propionic and acetic acids were estimated as 8·5 % and a considerable proportion of the butyric acid was recovered unchanged.

The precipitates of 2:4-dinitrophenylhydrazones from the non-volatile products were separated by recrystallisation from 96 % alcohol into two substances; one, melting at 291°, was recrystallised from ethyl acetate and chloroform and satisfactorily identified by comparison with a specimen prepared from methylglyoxal.

The other after several recrystallisations from alcohol melted at 201°. (Found (microanalysis): C, 42·97, 42·74; H, 3·72, 3·67; N, 19·45, 19·10 %. Molecular weight., by Rast's method, 279. $C_{10}H_{10}O_6N_4$ requires: C, 42·56; H, 3·55; N, 19·86 %. Mol. wt. 282.)

This hydrazone appeared therefore to be derived from a keto-derivative of butyric acid. Specimens of the corresponding hydrazones of α- and β-ketobutyric acids and of the half aldehyde of succinic acid were prepared: these melted respectively at 201°, 65° and 103°. However, on taking a mixed melting point of the unknown substance and the hydrazone prepared from α-ketobutyric acid, both melting at 201°, it was found to be 186°. Our supply of this substance was now exhausted and we were unable to explain the discrepancy. The α-keto-acid used for comparison was prepared by condensing together hippuric and pyruvic acids in the presence of acetic anhydride and decomposing the resulting azlactonecarboxylic acid by heating it with HCl.

Method of experiment.

In order to work with an approximately constant concentration of H_2O_2, the method of experiment finally adopted was as follows. A flask heated to 90° in a water-bath was fitted with an inlet tube (for admitting CO_2-free air) and with a reflux condenser connected to a series of flasks, the first of which was ice-cooled and contained water, and the remainder standard baryta. $N/35$ solution of the acid was introduced and the p_H adjusted to 6·4 by the addition of $N/2$ NaOH solution (bromothymol blue). The final volume was made up to 200 ml. and the oxidation started by adding 6 ml. of 20 vols. H_2O_2 solution and then, during 1 hour, hydrogen dioxide and $N/2$ sulphuric acid (or $N/2$ alkali as required) sufficient to maintain the concentration of dioxide at 0·19 % and the p_H at 6·4. During the course of the experiment a steady current of air was drawn through the apparatus. The rates of addition are shown in Figs. 1 and 2.

The amounts of hydrogen dioxide and acid to be added at any given time were found in preliminary experiments carried out in an open flask so that samples of the solution could be removed and analysed. Every 2 or 3 minutes if the action was rapid, or less frequently if it was slow, a drop of solution was removed by the pipette and the p_H determined using a bromothymol blue capillator; 1 ml. was also withdrawn and the hydrogen dioxide content determined by titrating with cold $N/20$ acid $KMnO_4$. Since in some of the experiments oxidation products were present which readily reduced the cold permanganate solution, the method was frequently checked by adding 1 ml. of the solution under examination to acidulated KI solution and estimating the iodine liberated.

These determinations can be rapidly performed and the necessary amounts of the dioxide and standard alkali added at frequent intervals to the main volume of solution. The amounts required were plotted against the time and s erved as a guide for the amounts of these reagents to be run in during subsequent

experiments; by repeated trial the amounts necessary to maintain a constant concentration of the dioxide and a steady p_H of 6·4 were arrived at.

When the oxidation had been continued for 1 hour, 10 ml. N alkali were added and the flask was rapidly cooled. The dioxide was quickly decomposed and sufficient $N/2$ sulphuric acid added to make the total quantity of inorganic acid added during the experiment just equivalent to the total amount of alkali; finally air was drawn through the apparatus for 20 minutes.

In the determinations which were made with the copper catalyst, before placing the acid to be oxidised into the flask, an aqueous solution containing 6 ml. of the 6 % hydrogen dioxide and 0·27 g. cupric sulphate crystals were introduced into the flask and the p_H of the solution was adjusted to 6·4 by the addition of $N/2$ NaOH; a brown precipitate formed and remained during the course of the experiment. Volatile products were removed from the reaction mixture by steam-distillation and the nature of the products in the residual solution was tested.

Rate of decomposition of hydrogen dioxide at 0·19 % concentration with and without copper catalyst.

When a solution containing 0·19 % H_2O_2 adjusted to p_H 6·4 was kept at 90° the concentration at the end of 1 hour was practically unchanged. If however 10 ml. of 2·73 % solution of cupric sulphate crystals and 4 ml. $N/2$ alkali (the quantity found necessary by trial to bring the p_H of the mixture to 6·4) were introduced into the flask and 200 ml. of the dioxide solution then added, the copper precipitate which had formed changed to a dark brown and remained undissolved. At first, the dioxide decomposed at the rate of about 0·2 g. per minute, 3·4 ml. of the 6 % dioxide being added each minute to maintain the concentration. Gradually the rate fell off probably owing to a surface change in the catalyst. The amounts of the dioxide added during 1 hour to maintain the concentration are plotted in Curve 0, Fig. 3; the rates of the decomposition of the dioxide after different intervals of time from the original formation of the cupric catalyst precipitate were obtained by ceasing to add the reagent, withdrawing 1 ml. of the mixture at intervals of 1 minute and determining its H_2O_2 content.

Thus directly after the formation of the copper precipitate the original concentration of the dioxide was reduced to 40 % in 3·3 minutes; samples tested after the precipitate had been formed for 4 hours, during which period hydrogen dioxide had been added to maintain the original concentration, showed that the concentration now fell to 40 % in 9 minutes, the activity of the catalyst having been considerably impaired.

Results.

Oxidation of the fatty acids in the absence of a cupric salt.

When a solution ($N/35$) of the sodium salt of a fatty acid was heated for 1 hour under the above-described conditions, no appreciable amount of decomposition of the dioxide was detected in the presence of the following acids: formic, acetic, propionic, butyric, hexanoic, β-hydroxybutyric and acetoacetic. With tartaric acid it was necessary to add about 15 ml. of the dioxide solution to maintain the concentration at 0·19 % during the hour's heating, an amount sufficient to oxidise the whole of the tartaric acid to CO_2. Some decomposition must however have occurred in the case of formic acid and to a less extent in the cases of propionic and of β-hydroxybutyric acids since the alteration in p_H showed that sodium carbonate had been formed from the salt of the acid.

These results are shown in Figs. 1 and 2; in Fig. 2 the amount of acid added to maintain the p_H at 6·4 was the measure of the sodium carbonate formed by the complete oxidation of the acid. Thus tartaric acid was completely oxidised

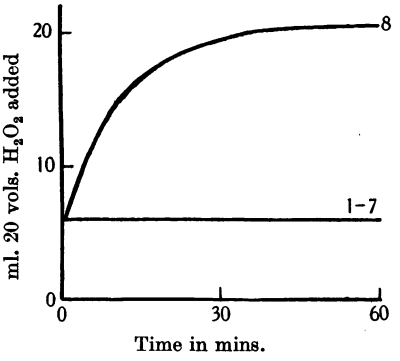

Fig. 1.

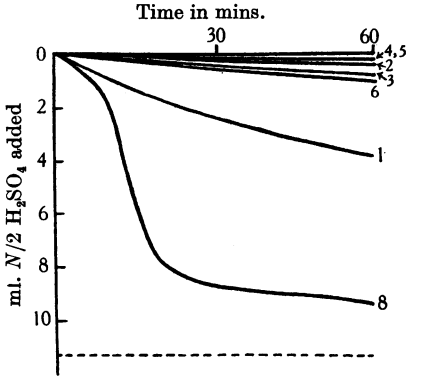

Fig. 2.

Figs. 1 and 2. Oxidation of fatty acids with hydrogen dioxide alone. 1, Formic acid; 2, Acetic acid; 3, Propionic acid; 4, Butyric acid; 5, Hexanoic acid; 6, β-Hydroxybutyric acid; 7, Acetoacetic acid; 8, Tartaric acid.

and about 40 % of the formate and less than 10 % of the propionate and hydroxybutyrate were converted into the carbonate. The amount of oxidation under the given conditions in the case of the other acids examined was insignificant.

Influence of the copper catalyst.

The addition of the catalyst produced a striking increase in the amounts of acids oxidised, as measured both by the quantity of hydrogen dioxide decomposed and by the changes in the p_H of the solution.

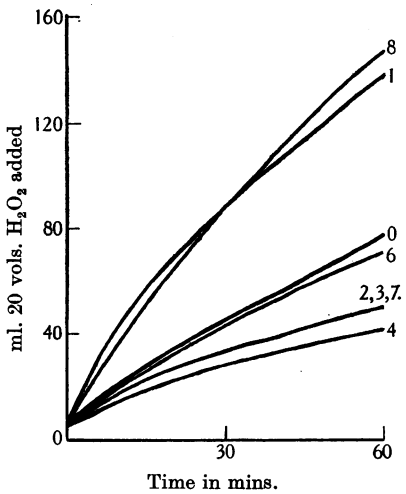

Fig. 3.

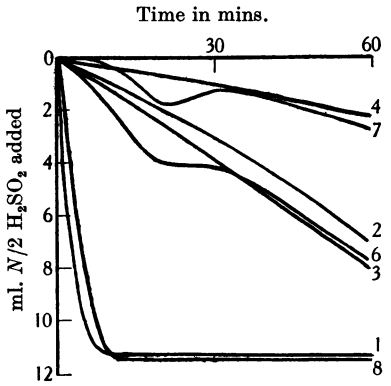

Fig. 4.

Figs. 3 and 4. Oxidation of fatty acids with hydrogen peroxide and a cupric salt. 0, Hydrogen dioxide alone; 1, Formic acid; 2, Acetic acid; 3, Propionic acid; 4, Butyric acid; 6, β-Hydroxybutyric acid; 7, Acetoacetic acid; 8, Tartaric acid.

Fig. 3 shows that the dioxide was most readily decomposed in the presence of the sodium formate and tartrate, the normal rate of decomposition of the dioxide being considerably increased. Acetic, propionic, butyric and acetoacetic acids all acted as inhibitors of the normal decomposition in spite of the fact that these acids were themselves being oxidised; β-hydroxybutyric acid was intermediate between the two groups, the rate of decomposition of the dioxide being almost unchanged by the addition of this acid to the solution. The difference between the actions of acetoacetic and β-hydroxybutyric acids was marked.

Examination of Fig. 4 which represents the additions of acid necessary to maintain a constant p_H brings out the following points.

Sodium formate and tartrate were rapidly converted into sodium carbonate, the formate being completely oxidised within 5 and the tartrate within 10 minutes: in order to maintain the constant p_H it was necessary to add sufficient acid to neutralise the theoretical quantity of sodium carbonate obtainable by the complete oxidation of these salts. The vigorous initial reaction which occurred in both cases probably accelerated the rate of decomposition of the dioxide.

Estimations of the carbonic acid liberated confirmed the complete combustion of these acids.

Sodium acetate, propionate and butyrate were not completely oxidised during the 1 hour for which the experiment was continued, the proportions of sodium appearing as carbonate being respectively 59, 68 and 18%: butyric acid was very much less oxidised than either propionic or acetic acid. Since only 18% of the sodium of the butyrate had been converted into carbonate, the remaining 82% must have been present as neutral sodium salts: propionic, acetic and succinic acids were identified and a considerable proportion of unchanged butyrate. Acetone was also present; its estimation at the end of the experiment showed that 28% of the butyric acid had been transformed into acetoacetic acid, but since the acetoacetic acid and acetone themselves undergo oxidation during the course of the experiment, the amount actually converted must have been considerably larger. The high percentage of acetone is in agreement with the results of Witzemann [1918] who showed that oxidation in a neutral medium favoured the production of acetone.

Table I. *Oxidation of 0·5 g. butyric acid (as sodium butyrate).*

CO_2		Acetone		Volatile acids		
Wt. g.	Percentage of total C	Wt. g.	Percentage of total C	Wt. Na salts, g.	Mean mol. wt. g.	Wt. sodium succinate, g.
(1) 0·236	23·6	—	—	—	—	—
(2) 0·250	25·0	0·097	22·2	0·18	73	0·027
(3) 0·288	28·8	0·088	20·0	0·20	77	0·059
(4) 0·281	28·0	0·087	19·8	0·17	74	0·032

Mean percentage of carbon			
As CO_2	As acetone	As volatile acid	As succinic acid
26·37	20·7	37·0	4·0

Oxidation of sodium acetoacetate and β-hydroxybutyrate.

The oxidation of acetoacetic acid was carried out by Dakin [1924] who mentions that he identified acetic, glyoxylic, formic and carbonic acids as products of its oxidation. Engfeldt [1921], using $KMnO_4$, found acetic, glyoxylic and oxalic acids, whilst Clutterbuck and Raper [1926], who worked at ordinary

temperature with hydrogen dioxide in strongly alkaline solution, obtained a number of products of which they believed $\alpha\beta$-dihydroxycrotonic or α-hydroxyacetoacetic acid to be the first formed. The influence of strong alkali in producing condensation products makes it improbable that this method furnished a useful analogy for the process of combustion of the fatty acids *in vivo*.

It seemed to us of some importance to compare the course of oxidation of β-hydroxybutyric and acetoacetic acids and to determine whether the hydroxy-acid passes through the keto-compound during its oxidation by means of the dioxide. The conditions used were the same as those described above with the addition of the cupric salt. Experiments were therefore carried out to find how rapidly the acetoacetic acid decomposed when kept under the conditions of the experiment without the addition of the dioxide: the results are shown in Table II.

Table II. *Rate of decomposition of acetoacetic acid in aqueous solution at* p_H *6·4 to 7·0 at 90°.*

		mg. acetone in 10 ml. of solution after heating		
			As acetoacetic acid	
mg. acetone as acetoacetic acid in 10 ml. of original solution	Duration of heating mins.	(*a*) Free	(*b*) Found	(*c*) Calculated for 11·1 mg. in original solution
11·1	0	0·0	11·1	11·1
11·6	5	1·13	10·5	10·0
11·6	10	2·01	9·62	9·2
11·1	15	2·65	8·15	8·1
11·1	25	4·27	6·87	6·9
9·1	40	5·50	3·53	4·3
9·5	50	6·18	3·04	4·0

In (*b*), the excess acetoacetic acid was determined as in Folin's method by drawing off the acetone from the salt-saturated solution.

Under the conditions of the experiment, even at 90°, the acetoacetic acid appeared to be only slowly decomposed into acetone and CO_2.

Curves (6) and (7) in Fig. 4 show that for the first 20 minutes a steady formation of alkaline carbonate took place, indicating either the decomposition of a corresponding amount of β-hydroxybutyric or acetoacetic acid into neutral substance (aldehyde or acetone) and alkaline carbonate or its complete oxidation to carbonic acid. The change in the slope of the curve for the next 10 minutes shows that excess of acid was being formed to neutralise the carbonate either by oxidation of a neutral substance or from the decomposition of the original acid into two acid molecules. During the second half hour the direction of the curve indicated a steady change from the sodium salt of an organic acid to sodium carbonate. Since it was probable from these data that the maximum amounts of neutral substance were present at the end of 20 minutes, an experiment was carried out for this period, the volatile products being passed into a solution of 2:4-dinitrophenylhydrazine hydrochloride. The hydrazones from the oxidation of 1·77 g. acetoacetic and from the same amount of β-hydroxybutyric acid were separately collected, dried to constant weight, the melting-points determined and the products crystallised. The results indicated that a maximum of about 13 % of the hydroxybutyric and 37 % of the acetoacetic acid had been converted into acetone. In both cases the hydrazones melted between 110° and 116° and consisted mainly of acetonehydrazone with a small amount of acetaldehyde-hydrazone. After several recrystallisations from alcohol, 80 % acetic acid was

used as the solvent, but acetone-2:4-dinitrophenylhydrazone appears to be rapidly decomposed by this solvent, acetyldinitrophenylhydrazine melting at 197° crystallising out.

Table III shows the amounts of CO_2 and acetone obtained after oxidising the hydroxy- and keto-acids for 1 hour under the given conditions. Nearly twice as much CO_2 was formed from the hydroxybutyrate as from the acetoacetate, but the proportion of acetone obtained from the latter was very much greater. It seems permissible therefore to conclude that under the given conditions the main path of oxidation of the β-hydroxybutyric acid does not pass through the keto-acid.

Table III. *The oxidation of β-hydroxybutyric and acetoacetic acids.*

Substance	Wt. acid taken g.	Wt. CO_2 g.	% C as CO_2	ml. *N* NaOH to neutralise vol. acids	Acetone Wt. g.	Acetone % of C
Na β-hydroxybutyrate						
Oxidised ½ hour	0·59	0·51	51·45	5·28	0·050	11·3
Oxidised 1 hour	0·59	0·77	77·1	4·5	0·022	5·0
„ „	0·59	0·74	73·7	1·7	0·029	6·6
Na acetoacetate						
Oxidised 1 hour						
Estimated by titration	0·61	0·45	45·4	2·5	0·110	24·8
Estimated by acetone	0·58					
„ „	0·58	0·42	42·3	5·4	0·09	20·9
Acetone						
Oxidised 1 hour	0·33	—	5·2	—	0·16	48·8

The oxidation of some possible intermediate products in the combustion of β-hydroxybutyric acid was investigated and the curves of the hydrogen dioxide decomposition and acidity changes were plotted. Figs. 5 and 6 represent the changes produced by the oxidation of acetone, acetic, glycollic, oxalic, malonic, lactic, pyruvic, β-hydroxybutyric and acetoacetic acids.

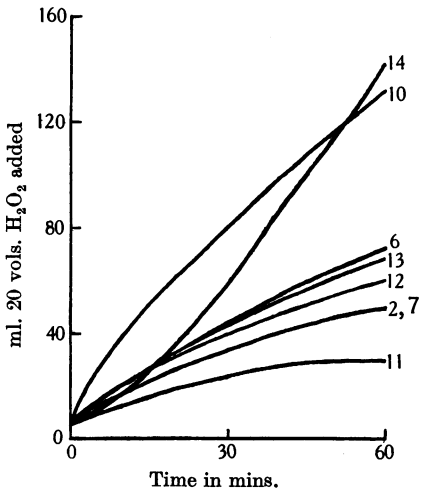

Fig. 5.

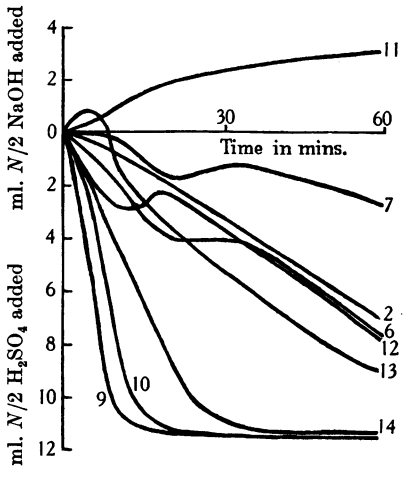

Fig. 6.

Figs. 5 and 6. Oxidation of possible intermediate products formed from acetoacetic acid and β-hydroxybutyric acid. 2, Acetic acid; 6, β-Hydroxybutyric acid; 7, Acetoacetic acid; 9, Oxalic acid; 10, Glycollic acid; 11, Acetone; 12, Lactic acid; 13, Pyruvic acid; 14, Malonic acid.

(1) The oxidation of acetone (Curve 11) was slow. Since no sodium was present in the original substance, there was no formation of alkaline carbonate and only about 35 % of the acetone was converted into fatty acid during the hour. Probably a slow conversion into acetic acid was taking place: very little CO_2 was evolved.

(2) Previous oxidation of the methyl group of acetic acid greatly increased the rate of further oxidation. Oxalic and glycollic acids were rapidly oxidised, as was also malonic acid (Curves 9, 10 and 14, Fig. 6).

(3) With pyruvic acid there was so rapid a development of carbonic acid that the addition of a small amount of alkali was necessary to maintain the p_H at 6·4. Subsequently the curve followed very much the course of that of acetic acid (Curve 13, Fig. 6).

(4) The curve for lactic acid showed a fairly rapid development of alkalinity and in these experiments a strong smell of aldehyde was noticed at the beginning of the oxidation. It seems probable that the lactic acid decomposed into acetaldehyde and formate, the latter being then oxidised to carbonate (Curve 12, Fig. 6).

The production of acetic acid by oxidation of the aldehyde overtook the formation of the carbonate and after about 20 minutes the curve closely resembled that plotted for the oxidation of acetic acid.

(5) Curve 6, Fig. 6, representing the oxidation of β-hydroxybutyric acid, showed a break similar to that obtained with lactic acid: it would be consistent with decomposition into acetaldehyde and glycollic acid, the latter being readily oxidised to carbonate and this being neutralised at first by the acid added and then by the acetic acid formed from the aldehyde.

(6) If the sodium acetoacetate (Curve 7, Fig. 6) had been rapidly oxidised to acetone and CO_2, rapid formation of carbonate would have followed and as the formation of acid from acetone is slow, the curve would have dropped rapidly and then slowly risen. Actually the formation of carbonate was considerably slower than with acetic acid, the slight subsequent rise suggesting oxidation of a neutral product such as acetone and the gradual oxidation of acetic acid.

Application of the results of oxidations in vitro *to the oxidation of the acetone bodies in the organism.*

The chief facts that have been established with regard to the fate of acetoacetic and β-hydroxybutyric acids in the body may be summarised as follows:

(1) β-Hydroxybutyric acid is burnt in the body with ease and only traces of acetoacetic acid or acetone are excreted in the urine after its injection [Mackenzie, 1902; Dakin, 1910; Blum, 1910].

(2) *d*-β-Hydroxybutyric acid is more readily burnt than the laevo-form, none of the former being excreted [Mackenzie, 1902; Marriott, 1914].

(3) Administration of acetoacetic acid leads to the excretion of *l*-hydroxybutyric acid (Marriott, Blum).

(4) Acetoacetic acid is readily reduced in liver perfusion experiments, by liver tissue and by yeast cells [Embden and Michaud, 1908; Friedmann and Maase, 1910; Dakin, 1910]; in the presence of sugar, about 80 % of added potassium acetoacetate was converted into *d*-hydroxybutyric acid [Friedmann, 1932]. The reverse change is less readily accomplished.

The results now presented show that when sodium β-hydroxybutyrate is oxidised with hydrogen dioxide in the laboratory under the prescribed conditions, only a small proportion of it is oxidised to the keto-acid, the main part

being rapidly broken down to CO_2 and water without passing through acetoacetic acid. Some acetic acid is formed but formic acid if produced would have been so rapidly oxidised under the conditions of the experiment that it would not have been detected in any significant quantity.

It appears therefore legitimate to conclude that the acetoacetic acid formed by the combustion of fatty acids in the body may be reduced to β-hydroxybutyric acid and this acid burnt without again passing through the stage of acetoacetic acid.

We know that formic and acetic acids are normal constituents of the urine [Schotten, 1882–83; Thudichum, 1856]. When doses of 20–25 g. of these acids were given by mouth, less than 20 % formic and 10 % acetic acid were excreted. Knoop and Jost [1924] found that the injection of β-hydroxybutyric acid was not followed by a rise of the blood lactic acid, yet after feeding with the same acid, lactic acid appeared in the urine. They considered that this elimination was to be attributed to a stimulation of the kidneys and not to a direct conversion of the hydroxy-acid into lactic acid by α-oxidation. No evidence of any other intermediaries formed in the combustion of the acetone bodies has been produced, though the existence of oxygen-containing derivatives of acetic acid seems probable.

Oxidation of the fatty acids in the presence of glucose.

The antiketogenic effect of glucose in the body has led various observers to study the effect of glucose on the oxidation of the fatty acids *in vitro* and particularly on the oxidation of acetoacetic acid.

Shaffer [1921] found that when hydrogen dioxide was added to a mixture of acetoacetic acid and glucose in alkaline solution, the acetoacetic acid disappeared rapidly at room temperature, the rate of disappearance increasing with the amount of glucose present and the degree of alkalinity. No similar reaction occurred with acetone, β-hydroxybutyric acid or butyric acid. It was suggested that two molecules of acetoacetic acid condensed with some oxidation product of the sugar, such as glycollic aldehyde, since this, like glucose, caused increased oxidation of the acetoacetate; the condensation product would then undergo oxidation [Shaffer and Friedmann, 1924]. Witzemann [1918] studied the oxidation of butyric acid under widely varying conditions and obtained the greater proportion of acetone the more nearly neutral the reaction mixture was kept. The acetone was more readily oxidised in an alkaline medium; the addition of glucose by developing acid oxidation products, increased the acidity of the solution and therefore the yield of acetone.

If acetoacetic acid in the body were reduced to the hydroxy-acid before it was oxidised and the effect of glucose was concerned with this reduction as in the experiments with yeast carried out by Friedmann [1932], the condensation of acetoacetic acid with an oxidation product of glucose would be without significance as to the fate of acetoacetic acid in the body.

Effect of the presence of fatty acids on the oxidation of glucose.

The oxidation of glucose was studied, the concentration of dioxide being maintained at 0·19 % and the p_H at 6·4.

When no copper salt had been added, the addition of the fatty acid to the glucose solution greatly inhibited the oxidation of the glucose. On Fig. 7, the amounts of hydrogen dioxide added to maintain the concentration at 0·19 % during the hour for which the experiment was carried out are shown. The total amount of decomposition of the glucose was small: it was diminished by the

addition of formic, acetic and β-hydroxybutyric acids and almost entirely inhibited by propionic, hexanoic and butyric acids. Fig. 8 which represents the amounts of acid and alkali added to maintain a constant p_H brings out the

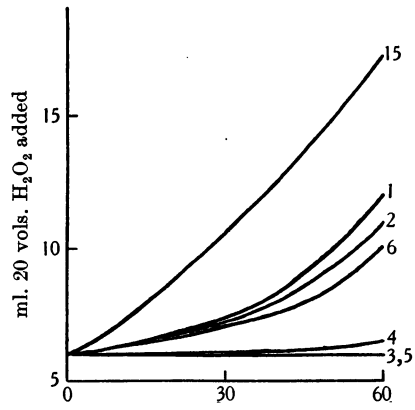

Fig. 7.

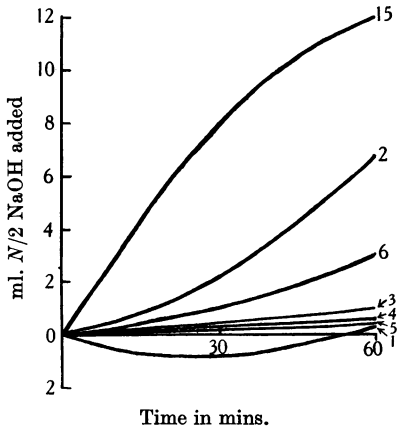

Fig. 8.

Figs. 7 and 8. Oxidation of fatty acids in the presence of glucose, without a catalyst. 1, Formic acid; 2, Acetic acid; 3, Propionic acid; 4, Butyric acid; 5, Hexanoic acid; 6, β-Hydroxybutyric acid; 15, Glucose alone.

same relationship. The presence of hexanoic, butyric and propionic acids almost entirely prevented the formation of acid products: with hydroxybutyric, acetic and formic acids the inhibition was partial. The degree of inhibition appears to be connected with the ease with which the acid undergoes oxidation, those acids which are least readily oxidised producing the strongest effect.

Oxidation of glucose and fatty acids in presence of a cupric salt.

The results of these experiments are represented in Figs. 9 and 10: the normal decomposition rate of the dioxide was greatly stimulated by the presence of glucose alone and glucose with formic acid. It was slightly inhibited by the addition to the glucose of acetic and β-hydroxybutyric acids, rather more when propionic and acetoacetic acids were added and very much inhibited by the presence of butyric and hexanoic acids.

In Fig. 10 the amounts of acid and alkali which were added to maintain the constant p_H are plotted. The acid products formed from glucose were first neutralised by additional alkali, and then as these were converted to carbonate, acid was added for its neutralisation. The time for the total oxidation of the glucose was slightly less than 30 minutes. Oxidation of the formate into carbonate was very rapid: this was then overtaken by the formation of acid products from the glucose, the whole action being over, as with the glucose alone, in about 30 minutes. The effects of acetic and hydroxybutyric acids were closely similar. Butyric and hexanoic acids strongly inhibited the combustion of the glucose.

The curves in Fig. 11 represent the results produced by subtracting the effect of the fatty acid alone (Fig. 4) from the effect obtained when it was added to the glucose (Fig. 10), thus the differences due to the conversion of the sodium of the original salts into carbonate are eliminated and the residual differences represent the degree of inhibition of the combustion of the sugar.

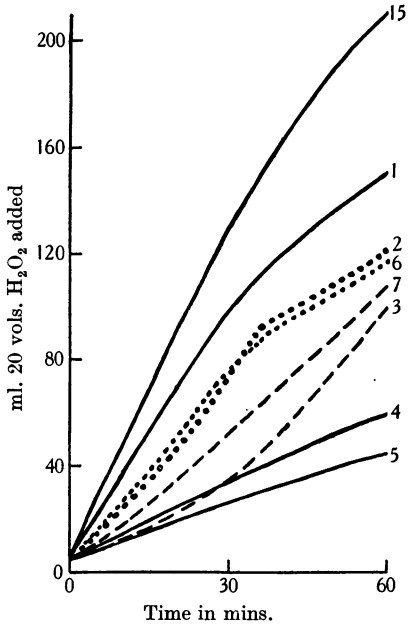

Fig. 9.

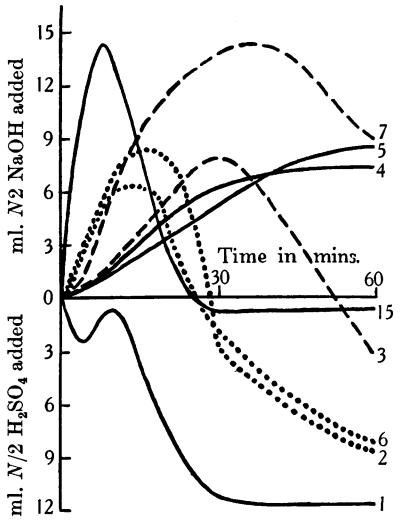

Fig. 10.

Figs. 9 and 10. Oxidation of fatty acids in the presence of glucose using a catalyst. 1, Formic acid; 2, Acetic acid; 3, Propionic acid; 4, Butyric acid; 5, Hexanoic acid; 6, β-Hydroxybutyric acid; 7, Acetoacetic acid; 15, Glucose alone.

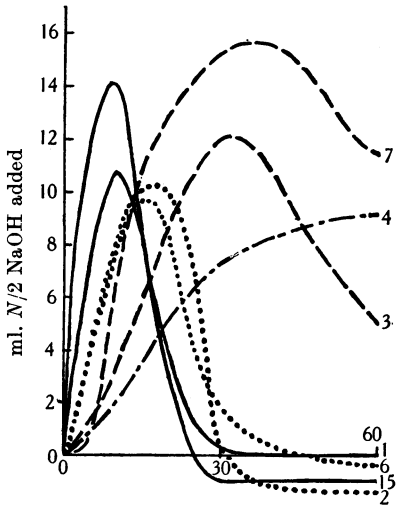

Fig. 11. Oxidation of glucose in the presence of fatty acids less the effect due to the acids alone. (Curves in Fig. 10 – curves in Fig. 4.) 1, Formic acid; 2, Acetic acid; 3, Propionic acid; 4, Butyric acid; 6, β-Hydroxybutyric acid; 7, Acetoacetic acid; 15, Glucose alone.

The time taken to reach the base line may be taken as the measure of the inhibition. The effect of the formic acid is very slight, those of acetic and β-hydroxybutyric acids are somewhat less; butyric acid is a very powerful inhibitor and acetoacetic and propionic acids are intermediate. The difference between the effects produced by β-hydroxybutyric and acetoacetic acids is very striking (Curves 6 and 7).

These results show that under the given conditions there is no evidence that the simultaneous oxidation of glucose increased the oxidation of the fatty acids investigated, but the fatty acids acted as inhibitors of the oxidation of the glucose by the hydrogen dioxide.

SUMMARY.

1. Oxidation of some lower fatty acids and their derivatives was effected by means of hydrogen dioxide at a temperature of 90°, the p_H of the reaction mixture being kept approximately constant at 6·4, and the concentration of the dioxide being kept as closely as possible at 0·19 %. The amount of oxidation was greatly increased by the addition of a cupric salt.

2. The substances investigated were formic, acetic, propionic, butyric, β-hydroxybutyric, acetoacetic, tartaric, glycollic, oxalic, lactic and pyruvic acids and acetone.

3. Under the given conditions only slow decomposition of the acetoacetic acid took place in the absence of the oxidising agent.

4. β-Hydroxybutyric acid was much more readily oxidised than acetoacetic acid and the main path of oxidation does not therefore pass through the keto-acid as a preliminary stage in oxidation.

5. Lactic and β-hydroxybutyric acids may suffer preliminary decomposition into acetaldehyde and a molecule of fatty acid.

6. The addition of the sodium salts of the fatty acids to a glucose solution inhibited to different extents the oxidation of the glucose, and the addition of the glucose did not promote the decomposition of the fatty acids.

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